Remarks

Claims 1, 6, and 38 have been amended without any intention of disclaiming equivalents thereof. Upon entry of this paper, claims 1, 6-23, 30-40, 42-56, and 117-123 will be pending and under consideration.

Claims 1 and 38 have been amended to recite a method for preparing a nerve graft *suitable for subsequent implantation* comprising degrading CSPG while maintaining basal lamina tube *structure*. Support for these amendment appears throughout the application and claims as filed, for example, in Figures 1A-1D; in Figure 19C; at page 9, lines 14-16; at page 15, lines 11-13; at page 42, lines 19-30; and in Example 4 of the application as filed. In addition, claims 1, 6, and 38 have been amended to correct grammatical errors. Applicant believes that the aforementioned amendments introduce no new matter. The outstanding objections and rejections are addressed in the order in which they appear in the Office Action.

Rejection Under 35 U.S.C. §112, Second Paragraph

According to page 2 of the outstanding Office Action, all pending claims presently stand as rejected under 35 U.S.C. § 112, Second Paragraph. Without acquiescing to this rejection but in order to expedite prosecution, Applicant has amended claims 1, 6, and 38 to delete "untreated" from the claims and to identify the reference graft as not having the specific characteristics of the prepared graft identified in the same claims. Accordingly, Applicant respectfully requests that this rejection be reconsidered and withdrawn.

Rejections Under 35 U.S.C. §102

The La Fleur Reference

Claims 1, 6-15, 17-21, 30-40, 42-51, 53-56, 117-120, 122 and 123 presently stand rejected as being anticipated by La Fleur *et al.* (J Exp. Med. 1996, 184:2311-2326, hereafter "La Fleur") under 35 U.S.C. §102(b). Applicant respectfully traverses this rejection in view of the present amendments and following remarks.

As an initial matter, La Fleur fails to teach or suggest *a method for preparing a nerve graft suitable for subsequent implantation*, which is the subject of Applicant's invention and is required by Applicant's claims, as amended. Instead, La Fleur's objectives include identifying

endogenous matrix metalloproteinases (MMPs) and the tissue inhibitors of MMPs (TIMPs) involved in repair after peripheral nerve injury. La Fleur's objectives also include determining the possibility that protection of basement membrane from proteolytic degradation is a relevant mechanism during repair of injury to nerve. (Page 2312, left column.) La Fleur observes that, in response to crush injury, TIMP-1 is induced. (Abstract.) La Fleur also observes that TIMP-1 protects basement membrane type IV collagen from degradation by exogenous MMP-9 in cryostat sections of nerve *in vitro. (Id.)* La Fleur concludes that TIMP-l may protect basement membrane from uncontrolled degradation after injury *in vivo. (Id.)* More specifically, La Fleur concludes with a proposal that in the proteolytic environment of injured nerve, TIMP-1 helps to preserve Schwann cell basement membrane during Wallerian degeneration, thus promoting axonal regrowth *in vivo*. (Page 2323, right column.) Accordingly, La Fleur fails to teach or suggest a *method for preparing a nerve graft suitable for subsequent implantation.* This is simply not a problem with which La Fleur is concerned.

In addition, La Fleur fails to teach or suggest a method of *degrading, by in vitro culturing, chondroitin sulfate proteoglycan of a nerve graft* as required by Applicant's claims, as amended. Specifically, Applicant's invention facilitates selective degradation and maintenance of components of the basement membrane of a nerve graft by *in vitro* culturing. In particular, the claims require degradation of CSPG while maintaining an intact basal lamina tube structure. *See, e.g.,* Figures 19A-19C; page 27, lines 20-23; page 55, lines 28-30; and Example 18. A similar phenomenon, Wallerian degeneration, is observed *in vivo* following nerve injury.

Whereas Applicant's claimed method requires *degrading, by in vitro culturing, chondroitin sulfate proteoglycan while maintaining an intact basal lamina tube*, La Fleur proposes that protection of the basement membrane from degradation promotes axonal regrowth in injured nerves, *in vivo*. (Page 2322, left column; page 2323, right column.) CSPG is a component of the basement membrane that is known to be degraded by MMPs. Krekoski *et al.* (2002) J. Neuroscience 22(23): 10408-10415 (IDS reference R25); Ferguson *et al.* (2000) Mol. Cell. Neurosci. 16: 157-167 (IDS reference R16). La Fleur indicates that TIMP-l preserves CSPG by protecting against MMPs during *in vivo* degeneration *(i.e.,* Wallerian degeneration). (Pages 2321-23.) Then, in her conclusion, La Fleur proposes that TIMP-1 protection against

degradation of basement membrane promotes axonal regrowth of an injured nerve *in vivo*. (Page 2322, left column, and page 2323, right column.) Accordingly, La Fleur fails to teach or suggest a method of preparing a nerve graft by degrading CSPG, as required to Applicant's claims.

Indeed, La Fleur not only fails to teach or suggest this element of Applicant's claims, but in proposing that preservation of CSPG promotes axonal regrowth, actually teaches against the present invention. Specifically, if La Fleur suggests anything about treating a nerve graft, it is that the entire basement membrane should be preserved. This is antithetical to Applicant's claimed invention, which requires selective degradation of CSPG while maintaining the basal lamina tube structure.

In the rejection, the Office Action references two analytical protocols in La Fleur (page 2312, column 2, paragraphs 1-2) in which segments of nerves are removed from animals and cultured. Office Action, page 3, last paragraph. The first protocol includes removing crushed or control nerve segments from mice and culturing them for 24 hours. The second protocol includes removing control nerve segments and culturing them with macrophage- or growth factor conditioned-media to test the effect on of these media conditions on TIMP-1 expression from the control nerve segments. Both protocols appear to include a final step of removing the nerve segments from the culture and placing them in TRIzol, which is a concentrated solution of phenol. TRIzol Material Safety Data Sheet, Invitrogen, Carlsbad, California, last revised 9/8/06 (IDS reference C5).

Applicant respectfully submits that these two methods for analyzing factors expressed from nerve tissue do not disclose or even suggest *preparing a nerve graft suitable for subsequent implantation*, as required by the Applicant's claims. In addition, there is no indication that these methods yield a nerve tissue sample with an *intact basal lamina tube structure*. Moreover, nerve tissue subjected to these methods could be harmful if implanted as a nerve graft. For example, these methods include, as a final step, submersion of the nerve tissue in TRIzol to extract nucleic acids from tissue samples. (Page 2312, column 2, second paragraph.) As noted above, TRIzol is a solution of concentrated phenol. Phenol is a toxic and corrosive material. *IPCS Environmental Health Criteria for Phenol (161)*, World Health Organization (publ. 1994, available at the web site, www.inchem.org/documents/ehc/ehc/ehc161.htm, last visited November 12, 2007) (first

draft by Montizan GK, printed in Finland) (IDS reference C6); Brooks and Riviere (1996) "Quantitative Percutaneous Absorption and Cutaneous Distribution of Binary Mixtures of Phenol and para-Nitrophenol in isolated Perfused Porcine Skin. Fundamental and Applied Toxicology" 32: 233-243 (IDS reference C7). Exposure to phenol is known to cause deep necrosis, cardiac dysrhythmias, metabolic acidosis, hyperventilation, respiratory distress, acute renal failure, renal damage, dark urine, methaemoglobinaemia, neurological effects (including convulsions), cardiovascular shock, coma and death. *See* IDS reference C6, Section 1.7. Accordingly, the use of concentrated phenol is incompatible with *preparing a nerve graft suitable for subsequent implantation.*

La Fleur also describes the isolation of proteins from a tissue sample through the homogenization of the nerve segment. (Page 2312, column 2, last paragraph.) As homogenization involves crushing and rendering the sample into a fine powder or slurry, this process also is unsuitable for a method for *preparing a nerve graft suitable for subsequent implantation*, and fails to *maintain an intact basal lamina tube structure*, as required by the present claims. Accordingly, the structure of a graft that undergoes the procedure described by La Fleur could not fulfill the requirements of the present claims. Instead, such a graft would contradict those requirements.

Therefore, Applicant submits that La Fleur fails to teach or suggest each and every element of claims 1, 6-15, 17-21, 30-40, 42-51, 53-56 117-120, 122, and 123 of the present invention, and respectfully request that this rejection be reconsidered and withdrawn.

The Lassner Reference

Claims 1, 6-15, 17-23, 30-40, 42-56 and 117-123 presently stand rejected as being anticipated by Lassner *et al* (J Reconstruct Microsurg 1995 11(6): 447-453, hereafter "Lassner") under 35 U.S.C. §102(b). Applicant respectfully traverses this rejection in view of the present amendments and following remarks.

Lassner describes methods for preserving peripheral nerve grafts. Specifically, Lassner describes methods for preserving cellular viability in peripheral nerve grafts. The paragraph bridging columns 1 and 2 of page 448 of Lassner describes three experimental groups: nerve

segments placed in cold storage at 4°C under ischemic conditions for periods of 14 hours, 32 hours, 72 hours, or 120 hours (Groups A-H); normal animal controls having the nerve dissected, left in the animal, and subsequently sutured in the absence of extracorporeal pretreatment (Group K), and negative controls where nerves were subjected to repeated freezing and thawing to evacuate all viable cells (Group I). Therefore, the nerve sections were (1) removed from the animal and stored in cold, ischemic conditions prior to implantation, (2) left in the animal after nerve dissection and subsequently sutured, or (3) removed from the animal and simply rendered acellular prior to implantation.

These activities do not rise to the level of *in vitro culturing*, as required by Applicant's claims. The cold, ischemic conditions are stasis conditions and do not promote physiological activity. In Applicant's method, if there is no physiological activity, there is no *degradation of CSPG* and, thus, no *enhancement of post-implantation axonal traversal of an interface between the nerve graft and host nerve tissue*. Page 56, lines 17-19. Thus, Lassner's stasis conditions are not culturing conditions, and therefore, they are not conditions permissive to degrade CSPG or enhancement of post-implantation axonal traversal of an interface between the nerve graft and host nerve tissue. Furthermore, leaving the severed nerve in the animal is not an *in vitro* treatment (it is an *in vivo* treatment) and, thus, cannot anticipate claim limitations that require culturing *in vitro*. In none of the three described dispositions of nerve segments does Lassner disclose or even suggest degrading CSPGs, axons or myelin by culturing a nerve graft *in vitro*.

The outstanding Office Action also identifies a second series of experiments in Lassner, on page 448, column 2, last paragraph. Office Action, page 4, last paragraph; page 10, second paragraph. In the second series of experiments, nerve grafts were prepared, dissected into small segments, placed in a culture dish containing Dulbecco's Modified Eagle Medium, and maintained at 5% $C0_2$/95% air for two days. The tissue segments were then evaluated morphologically, fixed with methanol at -18°C, and immunohistologically stained without any reimplantation. Thus, these experiments describe preparation of histological samples and do not describe a *method for preparing a nerve graft suitable for subsequent implantation* or a method for *enhancing the regenerative potential of a nerve graft*, as required by Applicant's claims. Additionally, the described conditions do not result in a graft that is structurally the same as the

graft prepared from Applicant's claimed method. Specifically, there is no indication that Lassner's samples comprise degraded CSPG or have an intact basal lamina tube structure. Nor does Lassner's histological preparation method result in the enhancement of *post-implantation axonal traversal of an interface between the nerve graft and host nerve tissue*. It simply cannot, because histological samples do not function as nerve grafts.

In addition, Lassner's histological preparation method described in the second series of experiments fail to *prepare a nerve graft suitable for subsequent implantation* as required by Applicant's claims, as amended. For example, the method described by Lassner involves fixation of specimens with methanol at -18°C. Page 148, column 2, last paragraph. Methanol is a toxic substance which according the manufacturer's Material Safety Data Sheet "cannot be made non-poisonous" Methanol Material Safety Data Sheet, Fisher Scientific, last revised 6/29/07 (IDS reference C8). Accordingly, the use of methanol is not compatible with preparing a nerve graft for subsequent implantation.

Therefore, Applicant submits that Lassner fails to teach or suggest each and every element of claims 1, 6-15, 17-23, 30-40, 42-56 and 117-123 of the present invention, and respectfully request that this rejection be reconsidered and withdrawn.

The Dennis Reference

Claims 1, 6-15, 17-21, 30-32, 34-40, 42-45, 47-51, 53-56, 119, 122, and 123 presently stand rejected as being anticipated by US 6,448,076 to Dennis *et al* (hereafter "Dennis") under 35 U.S.C. §102(e). Applicant respectfully traverses this rejection in view of the present amendments and following remarks.

Dennis describes a method of acellularization and does not include a *degradation step by in vitro culturing*, as required by Applicant's claims. Briefly, rat peripheral nerve segments are surgically removed, pinned at slack length within a culture dish, and immediately submersed in Dulbecco's Phosphate Buffered Saline (PBS). Then, the acellularization method is carried out at room temperature. (Column 3, lines 34-50.) These acellularized nerve grafts reportedly support axonal regeneration and allow end-organ reinnervation. (Column 6, lines 21-24.) With regard to amended independent claims 1 and 38, Dennis does not describe a selective *degrading step by in*

vitro culturing. In Dennis, the nerve is placed in PBS and then acellularization is carried out. There is no *in vitro culturing*.

Furthermore, Dennis fails to teach or suggest *degrading CSPG of the nerve graft while maintaining an intact basal lamina structure*. In fact, Dennis teaches just the opposite. Dennis states, "the acellularization method of the present invention ... preserves the basal lamina in order to maintain the appropriate molecular signals and adhesion molecules to enhance axonal regeneration." (Dennis, Column 6, lines 7-12; emphasis added.) Thus, while Dennis may describe maintenance of the basal lamina, Dennis fails to teach degrading CSPG. Dennis does mention a method of acellularization which removes the cellular elements from peripheral nerve tissue while leaving the endoneurial architecture intact" (column 2, line 22-24). However, Dennis' resulting graft is described as having the cells removed. It is not described as having *degraded CSPG* as required by Applicant's claims.

Still further, Dennis fails to teach or suggest *enhancing post-implantation axonal traversal of an interface between the nerve graft and host nerve tissue*. As explained in the present application, culturing conditions under certain circumstances activate CSPG-degrading enzymes and/or involve addition of CSPG-degrading enzymes. *See, e.g.,* Specification, page 27, lines 20-23; page 28, lines 18-20; and Example 3. These conditions enhance post-implantation axonal traversal of an interface between the nerve graft and host nerve tissue. Although Dennis may describe nerve grafts that support axonal regeneration, it does not follow that either degradation of CSPG or enhancement of post-implantation axonal traversal of an interface between the nerve graft and host nerve tissue occur. This is demonstrated, for example, in Applicant's Example 18. Example 18 and Figure 20A indicate that axonal regeneration into acellular nerve grafts is enhanced by *in vitro* predegeneration, but that axonal growth occurred within the basal lamina tubes in both the predegenerated and control (i.e., acellular but not predegenerated) conditions. Thus, it simply cannot be inferred from Dennis' description of a graft supporting axonal regeneration that CSPG is degraded or that post-implantation axonal traversal of an interface between the nerve graft and host nerve tissue is enhanced.

In addition, the chemicals used in the graft preparation method described in Dennis damage the graft's *tubular structure* that is maintained in Applicant's claimed invention.

Accordingly, Dennis' methods are not compatible with a method for *preparing a nerve graft suitable for subsequent implantation* as required by the Applicant's claims. For example, Dennis describes submersing tissue in a solution of glycerol, in a solution of sodium deoxycholate, and in a solution containing TRITON-X 100 (columns 3-4 and claims 1 and 5, for example). Glycerol has been investigated for use in preserving testicular tissue as an option in fertility preservation for pre-pubertal boys who will lose spermatogenic cells as a result of chemotherapy. Keros *et al.* (2005) Human Reproduction, 20(6): 1676-1687 (IDS reference C9). In the study, when glycerol was used, the structure of the basal compartment of the tubules was severely damaged. The ultrastructure of the cryopreserved samples as revealed by high resolution microscopy confirmed the findings. While glycerol is a widely used cryoprotectant for cryopreservation of cells, results indicate that it is not appropriate for preservation of tissues where structural maintenance is critical, such as with maintenance of basal lamina tube structure.

Previous researchers have studied the efficacy of using sodium deoxycholate and TRITON-X 100 in graft preparation. See Hudson *et al.* (2004) Tissue Engineering, 10 (9-10):1346-58 (hereafter "Hudson") (IDS reference C10); Sondell et al. (1998) Brain Res. 795:44-54 (hereafter "Sondell") (IDS reference 11). For example, Hudson, like Dennis, focused on the chemical acellularization of grafts but did not describe a degradation step via *in vitro* culturing. Briefly, Hudson sought to develop an optimized chemical acellularization method specifically for peripheral nerve tissue. Various detergents, concentrations and contact times were evaluated based on their ability to clear out cells and cellular debris while maintaining the critical extracellular matrix (or basal lamina) structure. The "optimized" protocol that was determined was then compared to existing acellularization methodologies, including Sondell's method, which, like Dennis' method, employs sodium deoxycholate and TRITON-X 100. Hudson found that the method employing sodium deoxycholate and TRITON-X 100 was effective at clearing out cells and debris but destroyed the basal lamina tube structure. Figure 5; page 1356 column 1, last sentence; and column 2, first paragraph.

Therefore, Applicant submits that Dennis fails to teach or suggest each and every element of claims 1, 6-15, 17-21, 30-32, 34-40, 42-45, 47-51, 53-56, 119, 122, and 123 of the present invention, and respectfully request that this rejection be reconsidered and withdrawn.

Rejections Under 35 U.S.C. §103(a)

Claims 1, 6-23, 30-40, 42-56 and 117-123 presently stand rejected under 35 U.S.C. §103(a) as being unpatentable over Dennis, La Fleur, Ide *et al*, Brain Research. 1983, 288:61-75 (hereafter "Ide") and Evans *et al*, Progress in Neurobiology, 1994, Vol. 43, pages 187-233 (hereafter "Evans"). As explained above, both Dennis and La Fleur fail to describe *degrading, by in vitro culturing, CSPG of a nerve graft.* In addition, the methods of both Dennis and La Fleur result in grafts that are structurally different from grafts produced in accordance with the present claims, and also involve chemicals that simply are not compatible with a *method of preparing a nerve graft suitable subsequent implantation.* Ide and Evans fail to cure the deficiencies of Dennis and La Fleur, alone or in combination.

Ide describes the influence of the basal lamina and living Schwann cells on nerve regeneration. The methods described in Ide focus solely on acellularization and do not involve selectively *degrading, by in vitro culturing, CSPG while maintaining an intact basal lamina tube structure*, as required by the present claims. In fact, Ide teaches away from the claimed invention by suggesting that there may be "specific substances responsible for supporting the regenerating axons" within the inner surface of the basal lamina, thereby stressing the importance of its complete preservation. (Abstract; page 71, column 1, last paragraph through column 2, first paragraph.)

Evans is directed to *in vivo* predegeneration. *In vivo* predegeneration involves an experimental model used to investigate the mechanisms and potential benefits of Wallerian degeneration, a process that occurs naturally in the body prior to nerve regeneration. The Evans technique involves transecting the donor nerve and leaving the segment within the animal to "predegenerate" for a period of time prior to harvesting to allow Wallerian degeneration to take place with subsequent proliferation of Schwann cells and removal of myelin debris. The "predegenerated" grafts are then used for implantation in a different recipient animal; outcomes were compared with freshly recovered nerves used as a graft (i.e., nerves that had <u>not</u> been predegenerated *in vivo*). (Page 209, column, 1 paragraphs 1-2.) Evans does not describe or suggest that a nerve graft can be predegenerated *in vitro*, that is, selectively degenerated in a dish while maintaining the basal lamina tube structure as required by Applicant's claims. In fact,

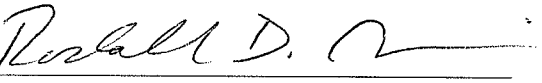
Evans teaches away from Applicant's claimed method in reporting that predegeneration has no clinical relevance. (Page 212.)

As explained above, Dennis and La Fleur do not describe *in vitro culturing* as required by Applicant's claims. Neither Ide nor Evans, alone or in combination, cures at least this deficiency. Accordingly, although Applicant submits that there is no reason why one of skill in the art would modify the teachings of Dennis and/or La Fleur as the Examiner suggests, and furthermore that there is no reasonable expectation of success were such modification to be undertaken, the fact is that Dennis, La Fleur, Ide and/or Evans fail to teach Applicant's claimed invention even when combined as the Examiner proposes. In view of the foregoing, Applicant respectfully requests that this rejection be reconsidered and withdrawn.

Conclusion

Applicant believes that, in the view of the above amendments and comments, the pending claims are in condition for allowance. Early favorable action is respectfully solicited. The Office is invited to contact the undersigned with any questions about this submission.

Respectfully submitted,



Date: November 12, 2007
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